XIV. *Further Notes on the Secretion of Potassium Hydroxide by* Dicranura vinula (imago), *and similar Phenomena in other* Lepidoptera. By Oswald H. Latter, M.A., Assistant Master at Charterhouse, formerly Tutor of Keble College.

Plates VIII. and IX.

In my previous communication on this subject (Trans. Ent. Soc. Lond., 1892, Part IV., pp. 287–292) a few points were left in doubt. Some of these I have been able to clear up, and at the same time I have proved that an alkaline fluid is produced from the mouth by *imagines* of other species at the time of their emergence from the cocoon. By the kind aid of my former colleague, the late Rev. S. D. Titmas, I have been able to make a quantitative analysis of the secretion produced by *D. vinula.*

I will deal with this species first and then proceed to the others, which I have not been able to investigate so fully.

(i.) *The cocoon of D. vinula.*

I am disposed to think that the difficulty experienced by the moth in escaping from the cocoon has been rather over-estimated. If a cocoon is examined from the innerside, while held towards the light, it will at once be seen that the walls are not of uniform thickness, but that thinner patches occur here and there. I examined over a hundred cocoons in this way, and invariably found one of these thinner areas at the anterior end opposite the head of the pupa. Such an arrangement obviously must lessen the labour of the imago at emergence.

(ii.) *The removal of the pupal " shield " by the imago.*

In my former paper I was unable to speak definitely of the manner in which the imago frees its head from

the shield. My friend, Mr. Archer Vassall, informed me, shortly after the publication of that paper, that he had observed the imago remove the shield by means of its front pair of legs. I have now myself repeatedly seen that this is the normal process. Almost as soon as the imago is free of the cocoon it halts for an instant, and raises its front pair of legs over its head, crossing the two tarsi dorsally to the shield; it then makes a rapid forward and downward stroke with the two legs simultaneously, and pulls the shield away, leaving it on the ground.

(iii.) *Mode of attacking the cocoon.*

In collecting the secretion for quantitative analysis, I was enabled to observe more fully the exact behaviour of the emerging imago. The pupæ were enclosed in glass tubes (to be more particularly described below) and were thus easily watched. The first violent struggles of the imago liberate its anterior regions from the pupa case, this latter is then thrust back a little until its posterior extremity rests against some solid resisting surface, which would in nature be formed by the posterior end of the cocoon. A series of peristaltic contractions then sweep over the abdomen of the imago from behind forwards, urging the head forward against the anterior end of the chamber until the pressure becomes considerable. Between each peristaltic movement the body slips backward a little by the partial telescoping of the abdominal segments. These movements constitute the strokes made by the labral prongs against the cocoon wall, and, at the same time, the muscular contractions compress the contents of the body and expel drops of potassium hydroxide from the mouth. If, after the imago has emerged, the action be imitated by gentle pressure between the fingers, more of the fluid can generally be driven out.

(iv.) *Quantitative analysis of the potassium hydroxide solution.*

The investigation of the strength of the solution proved difficult, and was attended by many failures. The following is the method which I found most satisfactory.

A large number of pupæ, due to emerge in about a fortnight's time, were placed in a corresponding number of glass tubes. The head of each pupa was placed so as to be in gentle contact with the closed end of the tube, while the remainder of the pupa was loosely wrapt in cotton wool to prevent violent movement, and also to absorb any excrement that might trickle downwards. Behind the pupa was placed a firm plug of cotton wool, in order to give it a solid object against which to thrust the discarded pupa case.

The tubes were arranged in rows on a board, which was slightly tilted, in order that the potassium hydroxide might flow down into the angle of the tube below the pupa head.

By careful watching, as the time of emergence drew near, I was able to collect a measurable quantity of the fluid from the tubes, and by instantly removing the moths and placing a pipette to their mouths a further quantity was obtained. Many tubes had to be rejected in consequence of fouling by excrement, in order that the fluid analysed might be absolutely pure. As the liquid was obtained it was placed in a small tube, previously carefully washed and dried, this was stoppered and kept in a corked bottle containing a little water, in order to prevent concentration by evaporation.

These precautions were necessary, as the period of emergence spread out over more than four weeks. The amounts obtained were dealt with in two lots, one lot amounted to 0·6 c.c., the other to 0·4 c.c. I did not count how many moths had contributed, for I was never able to obtain all the secretion of any one individual, and the computation would therefore be worthless.

The results of analysis (*vide* foot-note*) gave in the one instance 1·47 grammes, and in the other 1·40 grammes of potassium hydroxide in every 100 c.c. of the secreted liquid.

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* The method adopted was as follows :—The volume of secreted liquid was ascertained by scratching a mark on the tube at the surface of the liquid, and subsequently filling the tube up to the mark with water from a Geissler's burette, graduated to $\frac{1}{10}$th c.c., and provided with an Erdmann's float. The secretion was carefully washed out into a small beaker with distilled water, and the whole then faintly coloured with methyl-orange to serve as

(v.) *The origin of the potassium secretion*.

It will be best to trace the developmental changes which occur in the alimentary canal from the first days of pupal life up to the emergence of the imago, though the reverse order of proceeding was followed in the actual investigations, which extended over two summers. The digestive tract of a freshly formed pupa consists (Fig. 1) of a moderately long narrow œsophagus, followed by a long sacculated chylific stomach (mesenteron) with sacculated walls and of considerable width; this is succeeded by a straight narrow rectum, which is about half the length of the œsophagus. At the point of junction of the œsophagus and mesenteron there lies

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a delicate indicator. The burette was then filled with sulphuric acid of half-standard strength, *i.e.*, 24·5 grammes of $H_2 SO_4$ to 1000 c.c. of water; the dilute acid was then added drop by drop until the rosy-pink colour was just distinguishable. In the first experiment 0·6 c.c. of secretion was used : it required 0·32 c.c. of the dilute acid to produce the neutral condition (the second decimal place was estimated by the eye aided with a lens). Then the actual weight, *x*, of acid may be calculated thus—

$$1000 \text{ c.c.} : 0\text{·}32 \text{ c.c.} :: 24\text{·}5 \text{ gr.} : x \text{ gr.}$$
$$\therefore x = \text{·}0078 \text{ gramme.}$$

and taking the combining weights of the elements concerned as follows, viz., Hydrogen 1, Oxygen 16, Potassium 39, Sulphur 32 since 2 (KHO) neutralise $H_2 SO_4$

$$2 (39 + 1 + 16) \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad 2 + 32 + 64$$
$$112 \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \qquad 98$$

i.e., 98 grammes of sulphuric acid neutralise 112 grammes of potassium hydroxide, hence the weight, *y*, of potassium hydroxide present in the secretion can be found from the following statement—

$$98 : \text{·}0078 :: 112 : y.$$
$$\therefore y = \text{·}0089 \text{ gramme}$$

$\therefore$ 0·0089 gramme of KHO were present in 0·6 c.c. of secreted fluid

$\therefore$ in 100 c.c. of secreted fluid there would be 1.47 grammes of KHO.

In the second experiment 0·4 c.c. of secretion was taken and dealt with in precisely the same way. It required 0·2 c.c. of the dilute acid to produce the neutral condition in this case. Arguing as before, this gives 0·0056 gramme of KHO present in 0·4 c.c. of secretion, which, expressed in percentages, is 1·40 grammes of potassium hydroxide in every 100 c.c. of secreted liquid.

dorsally a minute diverticulum (*a r*), apparently springing from the anterior dorsal extremity of the mesenteron, but resembling rather the œsophagus in the character of its walls. The chief changes which take place concern this diverticulum, and the relative lengths of the three main sections of the canal. The latter may be dismissed in a few words—the œsophagus remains of the same length, the mesenteron becomes very much shorter and slightly narrower, while the rectum becomes correspondingly longer, until in the freshly-hatched imago it is rather longer than the two other sections combined, Figs. 2 and 3. There take place in the hinder region of the rectum other changes which I have not followed out in detail, the most remarkable of which is the existence, at the time of emergence, of a wide short tube (Fig. 3, *d*) opening into the body cavity at one end and into the posterior third of the rectum at the other; this tube is full of a brownish-red thick fluid (nitrogenous excretion ?), and appears to serve as an exit for the fluids so abundantly discharged *per anum* immediately after emergence. After four days of imaginal life the tube had shortened to a mere stump (Fig. 3A, *d*), and six days later it could not be recognized.

The mesenteric diverticulum gradually increases in size (*a r*, Figs. 2 and 3), and comes to lie in such a position that the only communication between the mesenteron and œsophagus is through its somewhat pointed ventral end. Eventually it assumes a pear-shaped appearance, with the broader end directed dorsally (Fig. 3A, *a r*).

At the time when the imago is ready to emerge this diverticulum contains a clear liquid with strongly alkaline reaction to red litmus paper; by gentle pressure the liquid may be driven along the œsophagus and caused to exude from the mouth in drops.

Now the contents of the larval mesenteron are intensely alkaline (as indeed is the case in every insect that I have examined), and it is probable that the digestive ferments need an alkaline medium for their action. Throughout pupal life the contents of the mesenteron continue strongly alkaline, though they undergo other alterations, viz., after fourteen days they are granular and blood-red in colour, and, as develop-

ment progresses, they become less granular and more transparent, while the colour changes to a greenish-black. The pigment, however, seems to cling to the walls, leaving the central portions of the fluid almost colourless.

It appears to me that we have here a fairly complete explanation of the somewhat astonishing behaviour of the imago in discharging an alkaline fluid. The digestive fluids of the larva are alkaline, their work ceases with the end of larval life, the walls of the mesenteron retain their power, and the alkaline liquid produced is retained and perhaps strengthened by concentration while being stored in the dwindled mesenteron itself and in the special receptacle constituted by the dorsal diverticulum.

I have not been able to analyse the alkaline liquid in the larval mesenteron in consequence of lack of material, so that the proof of the identity of the two fluids is not complete, though the balance of probability is in favour of their being one and the same.

There can be little doubt that the secretion is expelled along the œsophagus by means of the pressure which is brought to bear upon the mesenteron and its diverticulum, by the violent muscular contractions of the bodywall at the time when the imago splits open and struggles out of the pupa case.

(vi.) *Alkaline secretions discharged by other species.*

I have proved, by the employment of artificial cocoons of red litmus paper, that the following species effect a softening of their hard cocoons by discharge of alkaline liquid from the mouth :—*Dicranura bifida* (abundant), *D. furcula* (abundant), *Bombyx callunæ* (abundant), *B. lanestris* (slight), *Saturnia carpini* (abundant), *Limacodes testudo* (slight), and *Halias prasinana* (moderate quantity). I have not been able to procure sufficient material for analysis from any of these.

Pupæ of the following were also experimented with, but no results obtained—*Endromis versicolor*, *Pygæra bucephala*, *Mamestra brassicæ*, and *Cucullia absynthii*.

As in *D. vinula*, so too in most of the others which produce alkaline liquid, there are also mechanical con-

trivances to aid in rupturing the cocoon where this is necessary.

Both *D. furcula* and *D. bifida* emerge from the cocoon, wearing a " shield " similar to that formerly described in *D. vinula.*

D. furcula.

The pupal " shield " is shown in Fig. 4; it is held securely on the head of the imago by hooks fitting into grooves and sockets situated just to the median side of each eye of the imago (Fig. 4, *hk*). In addition to these hooks, there is also a pair of small processes projecting inwards from the inner ventral corner of the pupal eye (Fig. 4, *hk'*). I could not find any definite socket for the reception of these, but there is on the ventral surface of each side of the head of the imago a triangular depressed shallow area, in which this pair of hooks must lie while the " shield " is in position (Fig. 6, *hk'g*). After emergence the " shield " is removed by the tarsi of the front pair of legs, exactly as in *D. vinula.*

The labral prongs present in *D. vinula* are here represented only by short blunt knobs (Fig. 5, *l p*), and can be of no service in piercing the cocoon. I believe, however, that the maxillary palps are brought to bear upon the softened surface. These appendages are provided with far fewer and shorter hairs than in *D. vinula,* and are relatively stouter and harder; their distal extremities are entirely devoid of hair, sharply pointed, and marked with transverse ridges like a miniature file (Fig. 6, *p*). The structure of these palps appears to vary as to the number of joints. In three specimens both palps were single-jointed; in one specimen the right palp bore traces of three joints, but the left was single-jointed, while in another specimen both palps were distinctly three-jointed. The length of the palps is almost the same, viz., about 0·36 mm., whether single or three-jointed, and I am thus inclined to believe that a single-jointed condition is in process of evolution from the more primitive condition of three joints. Certainly the absence of intermediate articulations would render the palp a more rigid and efficient boring tool.

D. bifida.

Here the shape of the " shield " is slightly different, inasmuch as the posterior lateral edges of the pupal eyes are bent inwards, so as to partly grip the corresponding portion of the eyes of the imago. One pair of hooks are present on the inner face of the " shield," corresponding to the larger pair in *D. furcula,* and to the only pair in *D. vinula.* The smaller pair present in *D. furcula* here finds its homologue in a stout piece of chitin, which forms a low ridge merely. From the tips of the hooks long transparent chitinous threads pass upwards. The hooks fit into grooves and sockets in the head of the imago, as in the other species. This " shield " has also attached to it the chitinous lining of the mouth and œsophagus, which appear as transparent membranes. Traces of these organs can also be made out in the two other related species. The " shield " measures 2·2 mm. dorso-ventrally by 3·2 mm. transversely.

The head of the imago bears a pair of grooves (Fig. 7, *hkg*), leading into sockets for the reception of the hooks, but the labral prongs are hardly represented at all, a pair of minute lumps being the only vestige to be recognized. The remains of the mandibles (Fig. 7, *mn*) are rather prominent at the junction of the genæ with the inner border of the eye. The palps (Fig. 7, *p*), in the only two specimens I was able to examine, are moderately clothed with hair, except at the apex, which resembles that of *D. furcula.* Each palp consists of two well-marked joints, with indications of a possible third (Fig. 7, *p*). I would suggest that these are the organs by means of which the moth breaks open the cocoon.

Saturnia carpini.

The red litmus paper artificial cocoons for this species were made into a loosely coiled paper cone, the apex of which was left open and directed towards the light; the head of the pupa lay near the apex. I was greatly astonished to find an abundant alkaline secretion discharged prior to exit from the cocoon. Doubtless, the fluid softens the converging silk fibres at the neck

of the flask-shaped cocoon, and thus the labour imposed upon the moth is lessened. Judging only by the size of blue stain produced, the amount discharged is little less than that produced by *D. vinula.* The head of the moth and of the pupa present no modifications correlated with the possession of a hard cocoon.

Bombyx callunæ, B. lanestris, and *Limacodes testudo.*

These three species form a group by themselves, inasmuch as their cocoons have many points in common, and all three have similar appliances for escaping. The cocoons are all tough, more or less cylindrical with rounded ends, one of which is raised as a lid at the time of emergence. The boring organ is of a totally different kind to that existing in the Dicranurans, and is not formed either by labral prongs or modified maxillary palps, nor does the anterior portion of the pupa form a "shield" to the head and eyes of the imago. On the contrary, by carefully denuding the head by brushing and blowing, it may be seen that the head is far more turned down, so as to bring the mouth parts into a more backward position, while the median frontal portion of the head between the eyes is produced forward into a prominent and sharply-pointed umbo or boss (Fig. 9, *b*) of great strength, and capable of being used as a powerful awl in opening the lid of the cocoon. There are slight differences in the details observable in the three species named. *B. callunæ* and *B. lanestris* have the boss developed to a less degree and less sharply-pointed on the head of the pupa also (Fig. 8), while in *L. testudo* the converse holds good, the boss being far sharper and stronger in the pupa than in the imago (Figs. 10–12). Indeed, the pupal boss is the only hard structure on the otherwise fragile and delicate pupa case of this species. I am inclined to think that the lid of the cocoon is broken open before the imago of *L. testudo* has got free from the pupa case. I was unable to make any direct observations on this point, since the larva does not pupate till many weeks after the formation of the cocoon, the whole winter and early spring, in fact, being passed in the larval condition; and, further, the species is, in my experience,

very intolerant of the unnatural conditions attending
captivity.

Mr. Edmonds of Windsor informs me that they can
only be safely removed from their own cocoons just
before pupating, otherwise the larvæ endeavour to
construct a fresh cocoon, and so exhaust themselves.
He also tells me that in casting the last larval skin it
presses very hard on the sides of the cocoon and
accomplishes the moult with remarkable rapidity. If,
as is not improbable, strong pressure is applied to the
sides of the cocoon when the pupal skin is about to be
cast off, it can be readily seen that advantage would
result in the pupa being so armed with a pointed boss
that the exertion of bursting the pupa case should at
the same time drive the boss into the line of junction
between the lid and body of the cocoon. If this is so,
then, probably, the very slight alkaline effusion is of no
avail in softening the walls.

Halias prasinana.

I am indebted to the Rev. G. Chilton for pupæ by
means of which I ascertained that this species dis-
charges an alkaline liquid when escaping from the
cocoon. The amount produced is not great, but very
decided and more copious than that discharged by
B. lanestris or *L. testudo.* I could discover no definite
organ adapted for opening the cocoon, unless such be
represented by a pair of short sharp projections from
the anterior ventral margin of the rim of the eyes
(Fig. 14, *s*). It should be mentioned, however, that no
part of the cocoon is *removed* by the emerging imago.
The anterior portion of the cocoon is formed by the
drawing together of the two sides and the inter-
weaving of their component fibres, so that the imago
has merely to burst this suture and thrust the softened
walls apart right and left, the walls returning into
position immediately after. The mouth appendages are
undoubtedly inadequate for the work—the antliæ are
very long and flexible, lacking the necessary rigidity;
the palps are soft and terminate in remarkable sucker-
like discs (Fig. 13, *p*), they are curved upwards so as
to present their ventral surfaces to the front, the dorsal
surfaces being correspondingly concave; the former

surface is clad with long hairs (Figs. 13 and 14), the latter with ordinary scales, while the sides offer a very beautiful series, passing ventral-wards, showing the gradual modification of the ordinary scale into the long hairs (Figs. 15, i.–iv.). Situated just dorsal and slightly external to the insertion of the antliæ, and beneath the lateral edges of the labrum, is a pair of small appendages, consisting of a single joint only, and clad at their free extremities with a dense tuft of hairs (Fig. 13, *mn*) ; three fine fibres (muscular or tendinous) pass to each of these, two are inserted near the outer margin of, and one near the inner margin of the junction with the head. I could not detect any movable articulation, though the presence of these fibres would lead one to expect it. These appendages appear to me to be, without doubt, the mandibles. If this prove correct, it may throw light upon the systematic position of *H. presinana*.

I am aware that, in many particulars, these notes lack completeness ; I thought it best, however, to bring them together without further delay, in the hope that, by drawing attention to the secretion of alkaline liquids, others with more leisure for investigation than falls to my lot may be induced to take up and further extend the inquiries thus begun.